Microenvironmental Analysis of Biofilms

Part I - Microsensors

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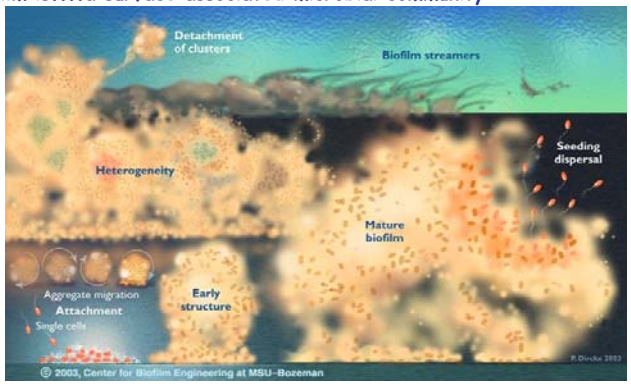
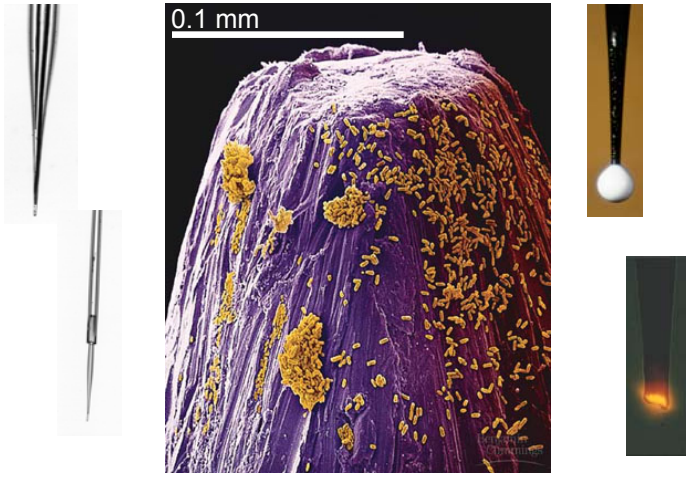
A biofilm is....a surface associated microbial community



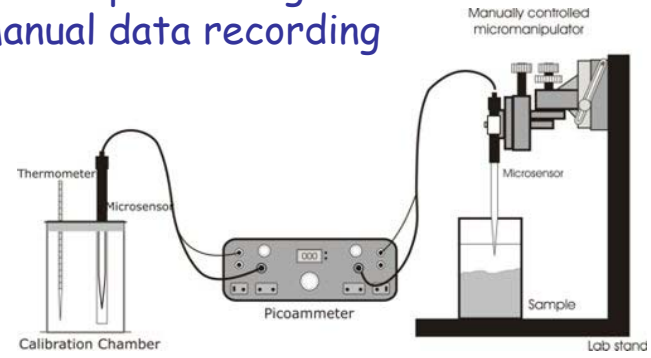
Biofilm communities represent a continuum of colonization, growth & production and detachment/degradation.

Measuring microbial activity at the appropriate scale ?

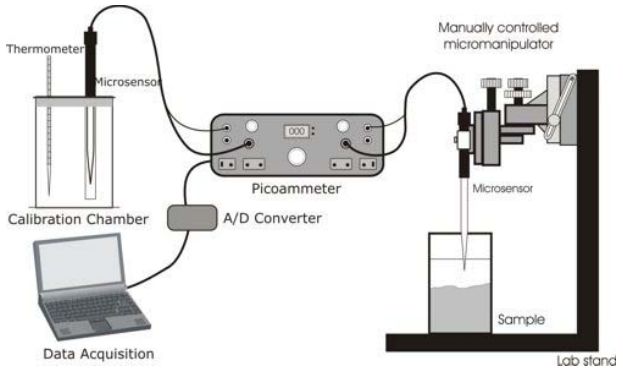


Simplest setup

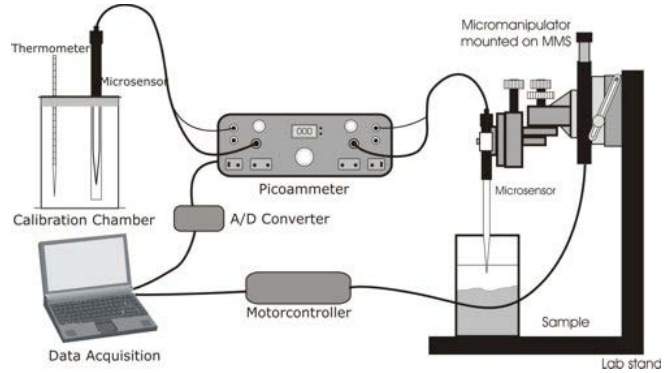
-manual positioning
-manual data recording



More fancy setup
-manual positioning
-automated data recording



Real fancy setup
-automatic positioning & data recording



Microsensors

Electrochemical

- *Potentiometric* sensors for: pH, CO_2, S^{2-} (solid state) pH, CO_3^{2-}, NO_2^-, NO_3^-, NH_4^+, Ca^{2+} (LIX-based)
- *Amperometric* sensors for: O_2, H_2S, N_2O, NO, H_2, H_2O_2 flow, diffusivity
- *Voltammetric* sensors for: Reduced Fe & Mn, O_2, S_{tot},...
- *Microbiosensors* for: NO_3^-/NO_2^-, CH_4, Glucose, BOD, VFA
- *Diffusivity* sensors

Kühl & Revsbech 2001, Revsbech 2005,Kühl 2005

Potentiometric microsensors - pH

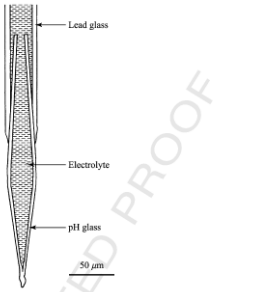


FIG. 2. Tip of glass pH microsensor. The cone made from pH-sensitive (i.e., hydrogen ion exchange) glass will integrate ambient pH over the entire length.

Integrated response over the exposed surface of the pH sensitive glas.

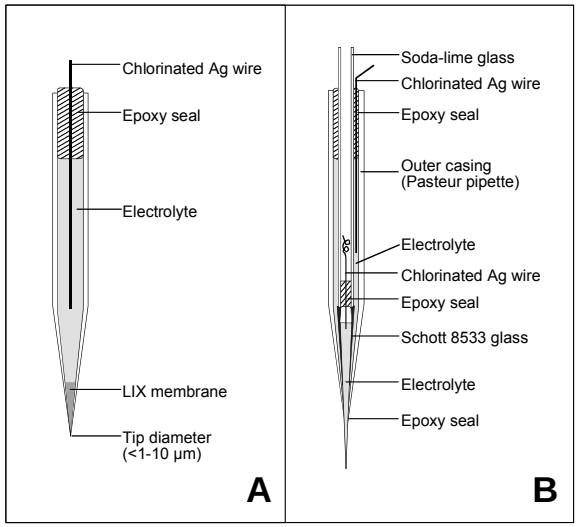
Fragile.

Potentiometric microsensors measure a Log-linear response of signal (mV) vs. Activity of ion.

At low ionic strength activity=concentration, but at higher ionic strength?

Revsbech 2005

Potentiometric microsensors - LIX



H^+
Ca^{2+}
NH_4^+

NO_2^-
NO_3^-
CO_3^{2-}

PO_4^- ?
SO_4^{2-} ?

Short life

Drifting signal

Interferences

Kühl & Revsbech 2001

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Kühl & Revsbech 2001, Revsbech 2005,Kühl 2005

Amperometric Clark-type O_2 microsensor

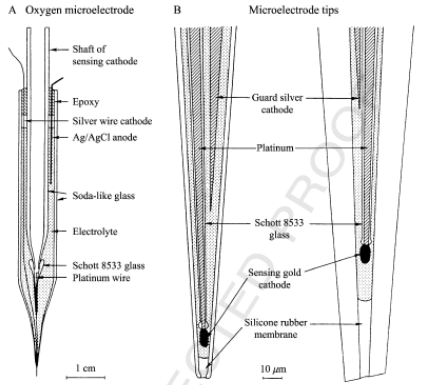


FIG. 1. Oxygen microsensor of the micro-Clark type shown with both a thin tip, which can be used for analysis where minimum sample disturbance is essential, and a sturdy tip for use in media that might otherwise cause sensor damage. The internal guard cathode removes oxygen from the internal electrolyte that might otherwise interfere. From Revsbech (1989), with permission from Limnology and Oceanography.

"Ideal" sensor

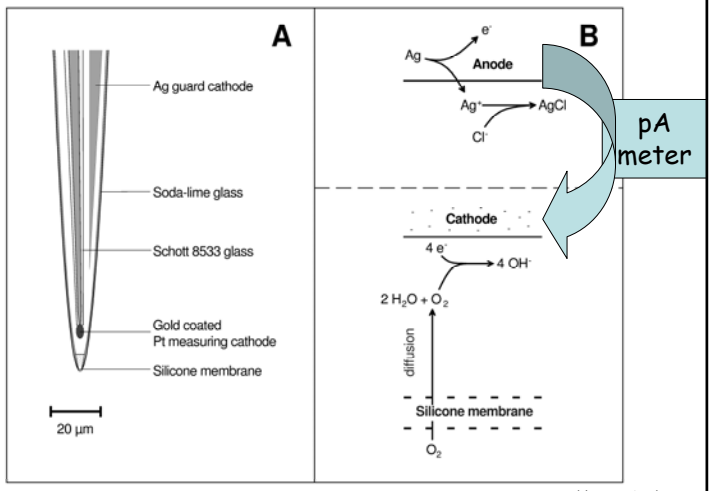
Low O_2 consumption
/Low stirring artefact

Linear, stable and fast response
(down to <0.2 s)

Taylorized performance
by varying tip dimensions

Revsbech 2005

Clark-type O_2 microsensor



Kühl & Revsbech 2001

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Kühl & Revsbech 2001, Revsbech 2005,Kühl 2005

Nitrate/Nitrite micro-biosensor

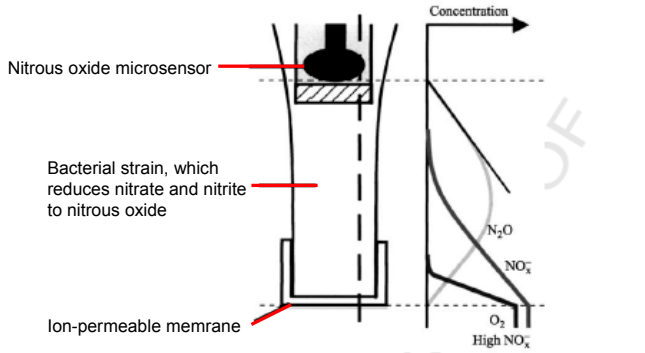

FIG. 6. Microscale biosensor for NO_2^- or NO_x^-. Bacteria in the tip convert incoming NO_2^- or nitrite plus nitrate (NO_x^-) to N_2O, which is subsequently monitored by the internal N_2O microsensor.

Microengineering using knowledge of diffusion, reaction kinetics and microbiology

Revsbech 2005

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Fiber-optic

- *Microprobes* for:
  - radiance, irradiance, scalar irradiance (UV-NIR light)
  - Surface detection
  - Pigment fluorescence
  - Diffusivity/Flow
- *Micro-opt(r)odes* for:
  O_2, pH, CO_2, temperature

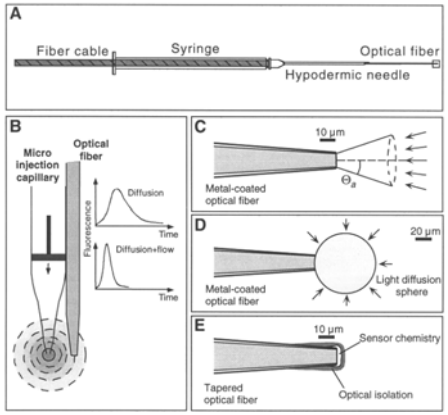
Kühl & Revsbech 2001, Revsbech 2005,Kühl 2005

Fiber-optic Microsensors

Microprobes (A-D) for:
- radiance, irradiance, scalar irradiance (UV-NIR light)
- Surface detection
- Pigment fluorescence
- Diffusivity/Flow

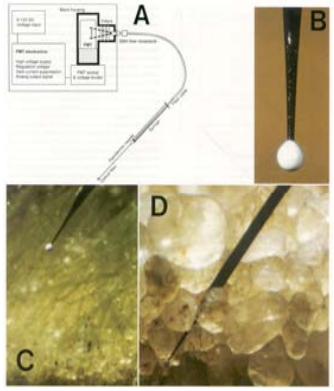
Micro-opt(r)odes (E) for:
- O_2, pH, CO_2, temperature

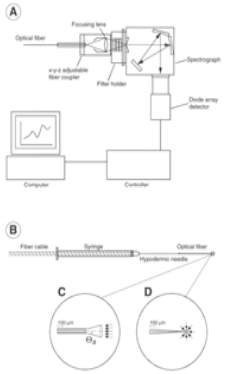
All based on multimode graded index optical fibers
100/140 µm core/cladding
N.A. = 0.22



Kühl & Revsbech 2001

Microscale light measurements

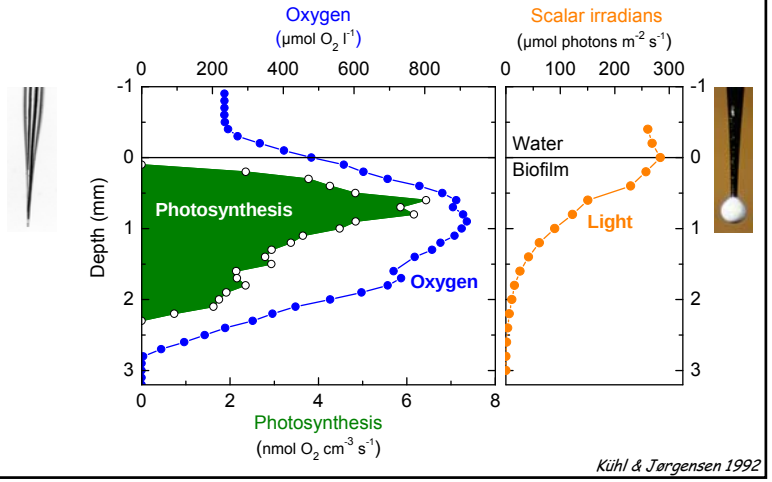




Kühl et al. 1997

Kühl & Jørgensen 1992.

Light and Photosynthesis



Kühl & Jørgensen 1992

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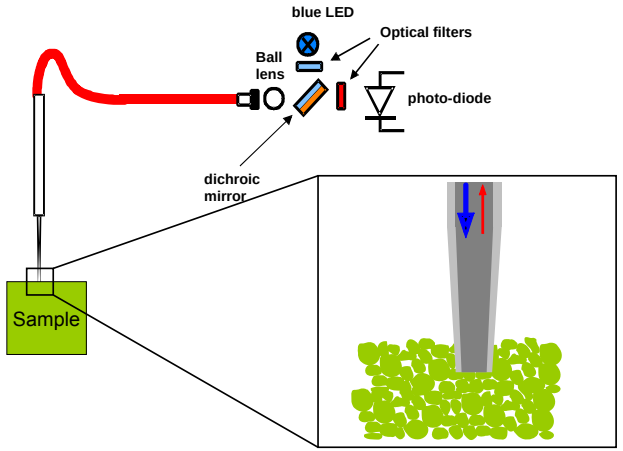
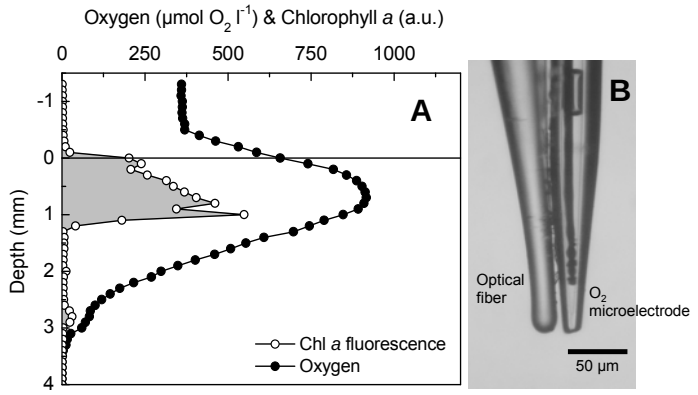
  - Surface detection
  - Pigment fluorescence
  - Diffusivity/Flow
- *Micro-opt(r)odes* for: O_2, pH, CO_2, temperature

Microscale fluorescence measurements



Combined oxygen and fluorescence analysis



Kühl 2005

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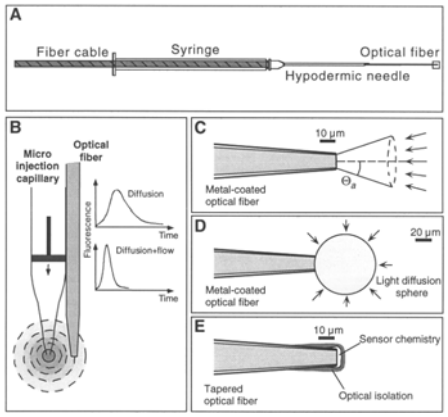


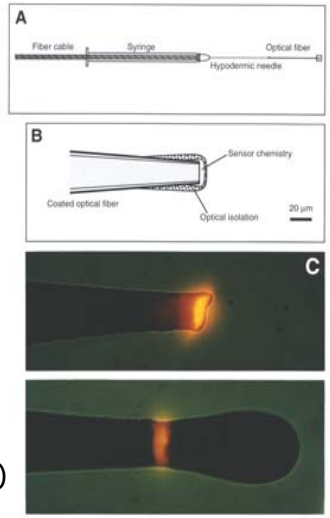
Kühl & Revsbech 2001

Micro-Optodes

Analyte dependend change in optical properties of an immobilized indicator dye.

Mostly based on dyes changing absorbance or luminescence.

Immobilization in various polymers
(PVC, polystyrene, sol-gel.....)



Klimant et al. 1995

Optical oxygen measurement

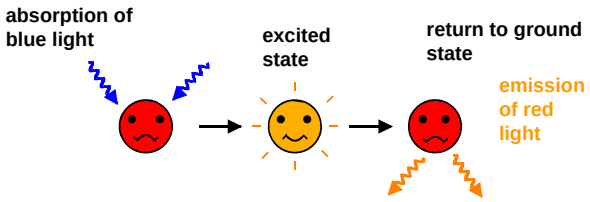
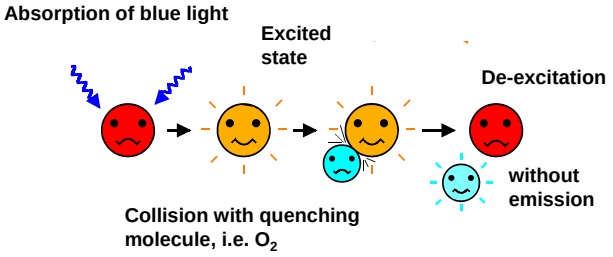


Photo-Luminescence of Ruthenium dyes & Platinum or Palladium porphyrins

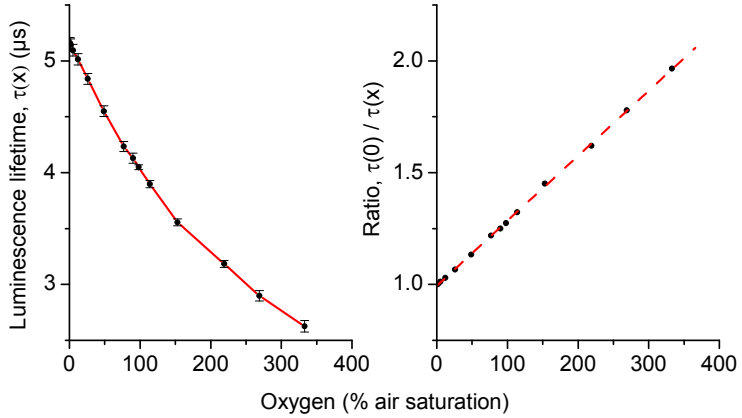
Optical oxygen measurement

Indicator luminescence is dynamically quenched by O_2



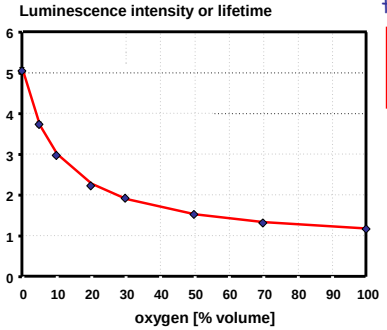
Ideal Stern-Volmer quenching response

$$\frac{I_0}{I(x)} = \frac{\tau_0}{\tau(x)} = 1 + K_{SV} \cdot [O_2]_x$$



Kühl et al. 2007

Calibration



Modified version of the Stern-Volmer equation:

$$\frac{I}{I_0} = \frac{\tau}{\tau_0} = \frac{1-\alpha}{(1+K_{SV}\cdot[O_2])} + \alpha$$

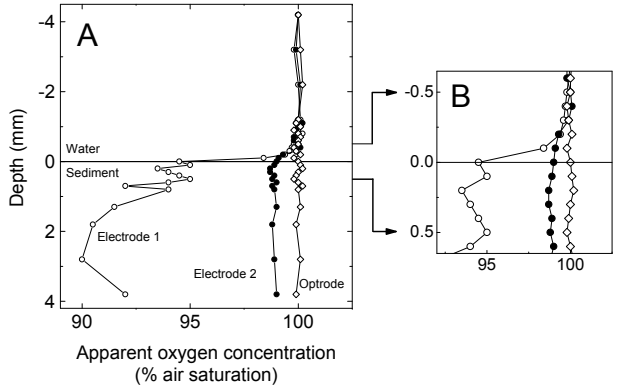
$$c = \frac{(I_0 - I)}{K_{SV}\cdot(I - I_0\alpha)} = \frac{(\tau_0 - \tau)}{K_{SV}\cdot(\tau - \tau_0\alpha)}$$

$$K_{SV} = \frac{I_0(c_2 - c_1) - (I_1 c_2 - I_2 c_1)}{(I_1 - I_2)\cdot c_1 \cdot c_2}$$

$$\alpha = \frac{I_1(1 + K_{SV} c_1) - I_0}{I_0 \cdot K_{SV} \cdot c_1}$$

Kühl 2005

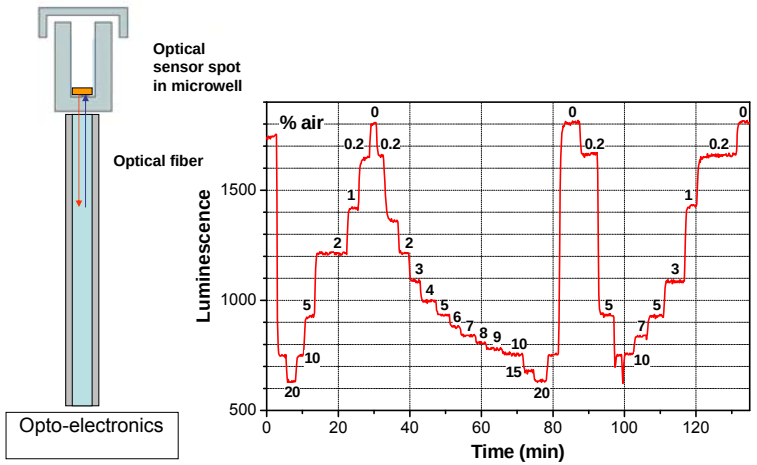
Oxygen optodes do <u>not</u> consume O_2



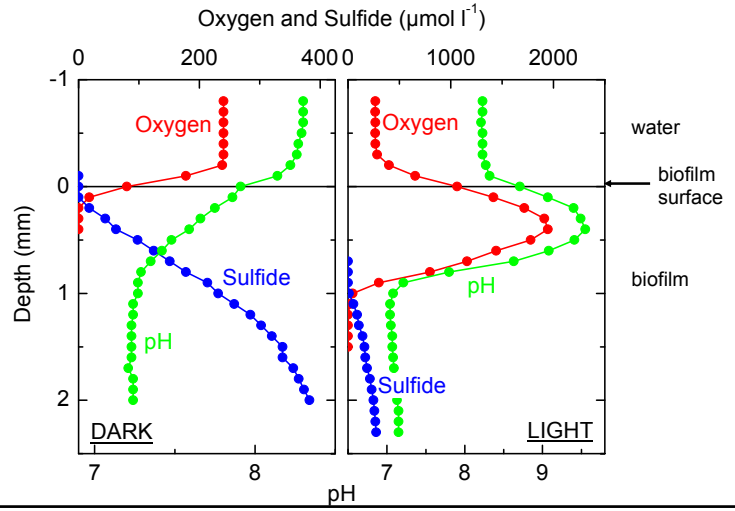
- And are not sensitive to sulfide ☺

Klimant, Mayer & Kühl 1995

Fiber-based (or plate reader based) respirometry in microtiter wells (Silicone + Pt-porphyrin)



How to read concentration profiles?

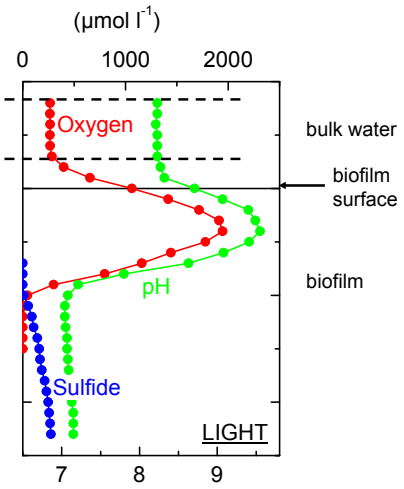


1. Bulk water

advective & turbulent mixing (also called "Eddy diffusion")

→For microsensor applications we can often assume constant concentration values in the bulk water

→Provides often easy calibration of e.g. oxygen microsensors



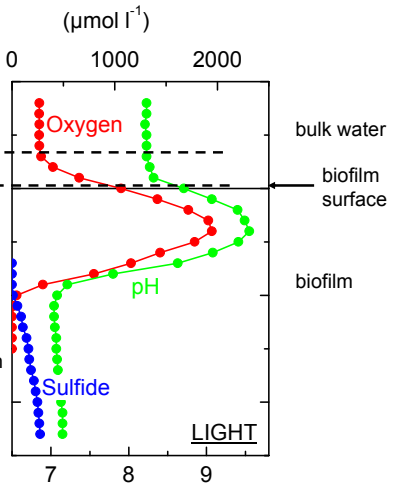
2. Diffusive boundary layer (DBL)

Diffusive boundary layer

Laminar flow along the biofilm surface.

→ horizontally: advective transport

→ vertically: pure molecular diffusion



The DIFFUSIVE BOUNDARY LAYER (DBL)

Exchange of solutes between water and sediment is due to a combination of turbulent transport (**eddy diffusion**, E) and **molecular diffusion**, D:

$D_{tot} = E + D$

In turbulent water E>>D.
Turbulences are dampened close to structures until E<D.

In a 0.1-1 mm thin water layer (DBL) just above the structure, solute tranport becomes dominated by molecular diffusion.

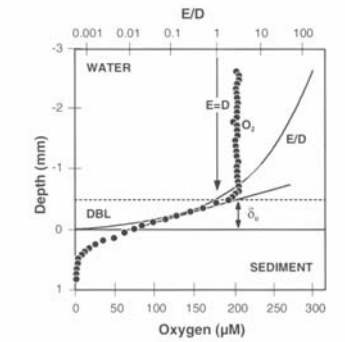
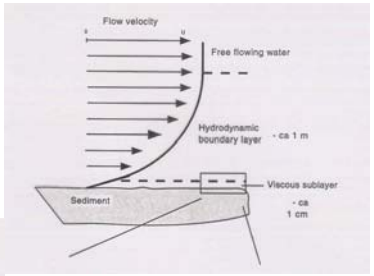
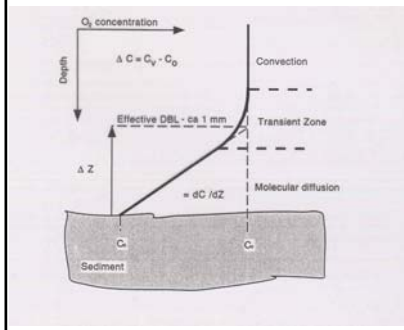


Fig. 5.5 Oxygen microgradient (n) at the sediment-water interface compared to the ratio, E/D (logarithmic scale), between the vertical eddy diffusion coefficient, E, and the molecular diffusion coefficient, D. Oxygen concentration was constant in the overflowing seawater. It decreased linearly within the diffusive boundary layer (DBL), and penetrated only 0.7 mm into the sediment. The DBL had a thickness of 0.45 mm. Its effective thickness, δ_e is defined by the intersection between the linear DBL gradient and the constant bulk water concentration. The diffusive boundary layer occurs where E becomes smaller than D, i.e. where E/D = 1 (arrow). Data from Aarhus Bay, Denmark, at 15 m water depth during fall 1990 (Gundersen et al. 1995).

Diffusive boundary layers surround all surfaces and often limit mass transfer.

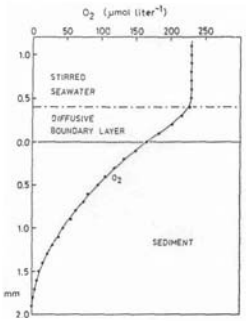


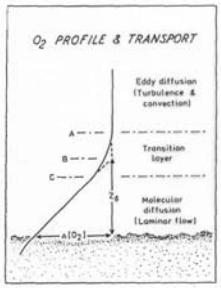


$$J = D_0 \frac{dC}{dz}$$

$$J = \frac{D_0}{\Delta z}(C_v - C_0) = k(C_v - C_0)$$

The DBL as a barrier towards solute exchange ?

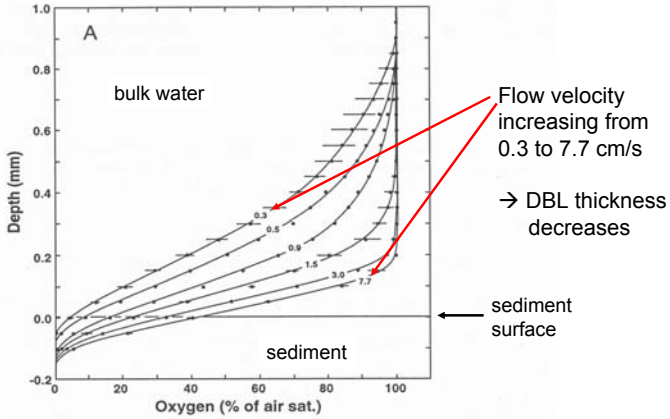




$$t \sim \frac{L^2}{2D_o}$$

Distance	- diffusion time O_2
1 μm	- 0.3 msec
100 μm	- 3.0 sec
1 mm	- 4.0 min
1cm	- 7.0 h
10 cm	- 25.0 d

The flow velocity of the bulk water determines the thickness of the DBL

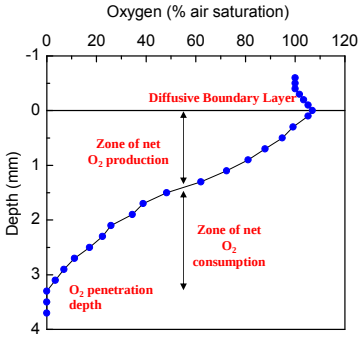


Jørgensen (2001)

Simple Oxygen Profile Analysis

Fick's 1'st law:

$$J(x) = -\phi D(x)\frac{dC(x)}{dx}$$



$\Rightarrow netto\_production$

Profile concave from left,

$$\frac{d^2C(x)}{dx^2} > 0 \Rightarrow R(x) - P(x) > 0$$

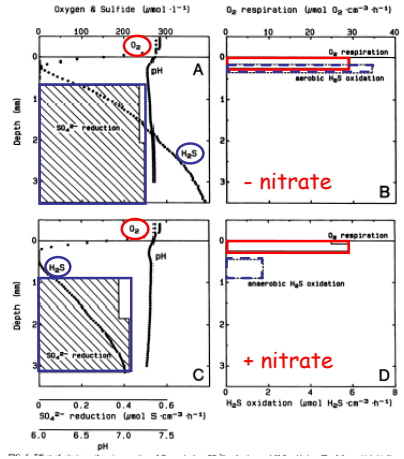
$\Rightarrow netto\_consumption$

Profile straight line,

$$\frac{d^2C(x)}{dx^2} = 0 \Rightarrow R(x) - P(x) = 0$$

$\Rightarrow pure\_diffusion$

Sulfur cycling in a biofilm



Fick's 2nd law:

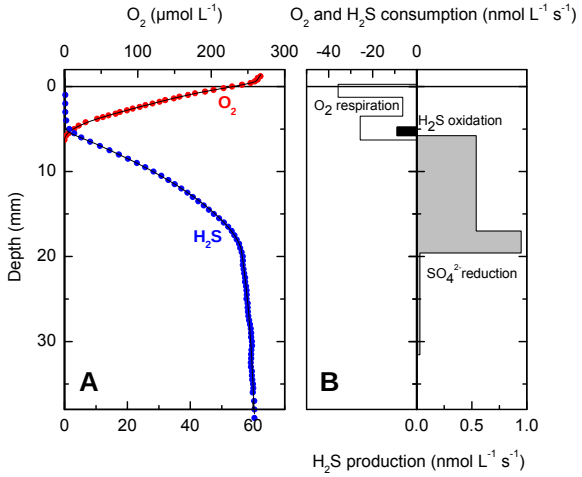
$$\frac{dC(x,t)}{dt} = D(x)\frac{d^2C(x,t)}{dx^2} - R(x,t) + P(x,t)$$

At steady-state dC/dt=0 causing:

$$P(x) - R(x) = -D(x)\frac{d^2C(x)}{dx^2}$$

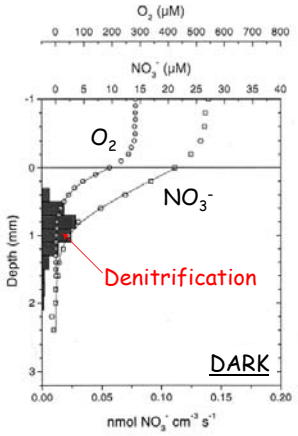
Kühl & Jørgensen 1992

Sulfur cycling in acidic lake sediment



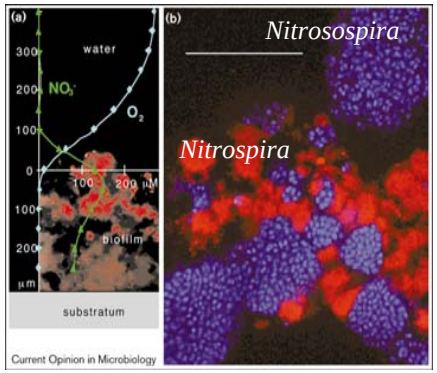
Kühl et al. 1998

Nitrogen cycling in diatom sediment



Combination of Microsensor Analysis and FISH in nitrifying biofilm

Nitrobacter



Examples of fluorescence *in situ* hybridization techniques.

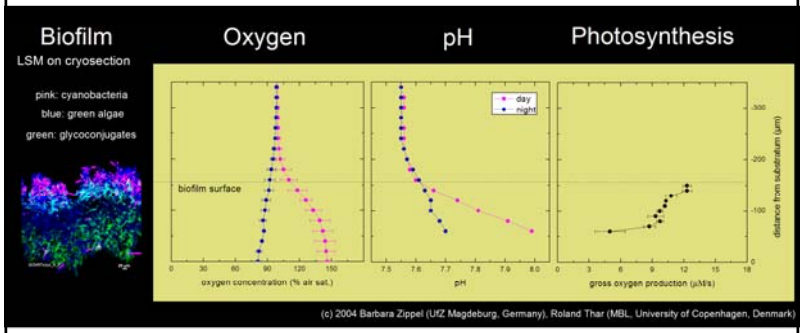
(a) *In situ* hybridization combined with microsensor measurements. *In situ* hybridization of a vertical biofilm slice with a carboxytetramethylrhodamine-labeled probe (NIT3) specific for the genus *Nitrobacter* (red stain cluster) correlated to oxygen and nitrate gradients measured by microelectrodes. Magnification, ´400. Adapted from Schramm *et al.* [63].

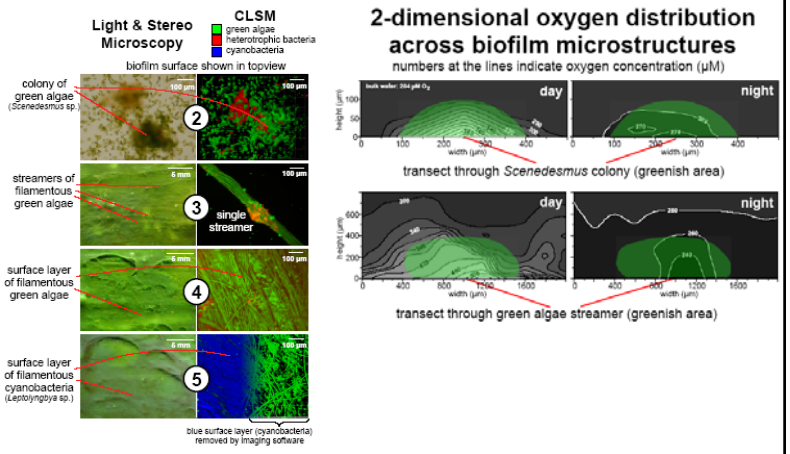
(b) Confocal microscopic image of a bacterial aggregate thin section after simultaneous hybridization with a Cy3-labeled probe specific for nitrite-oxidizing *Nitrospira* sp. (red) and a Cy5-labeled probe specific for ammonia-oxidizing *Nitrosospira* sp. (blue). Scale bar indicates 20 mm (A Schramm, M Wagner, unpublished data).

$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$

Amann&Kühl 1998

Combination of Microsensor Analysis and CLSM





Thar & Kühl in prep.

Microsensors

Advantages:

☺ Miniature instruments with electrochemical or optical detection principle

☺ 1-100 times thinner than a hair (0.001-0.1 mm tip diameter)

☺ Fast and specific response to analyte (t_{90} <0.2-60 s)

☺ Commercially available

Disadvantages:

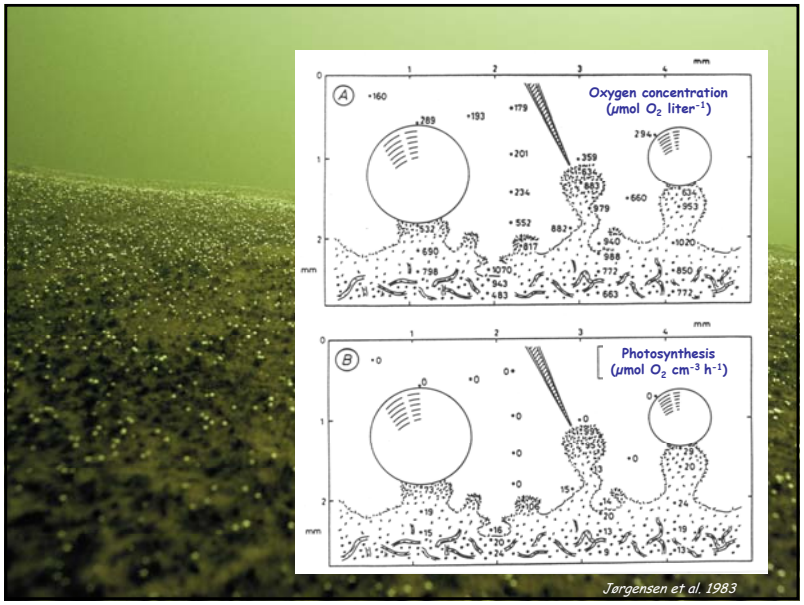
☹ Fragile and time consuming to prepare

☹ **NOT** non-invasive!!!!

☹ Point measurements

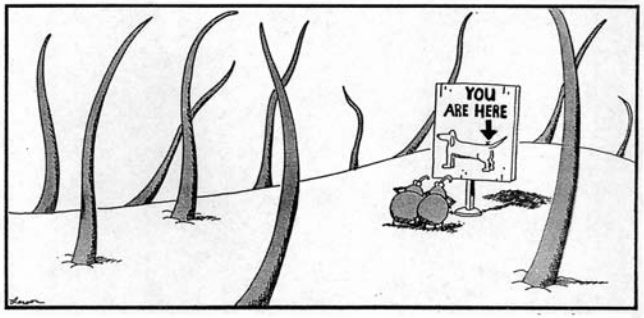
Microenvironmental Analysis of Biofilms

Part II - Imaging



Jørgensen et al. 1983

Interfaces and Microenvironments



Spatial and Temporal Heterogeneity

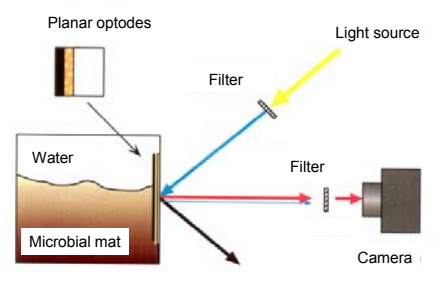
Optodes for 2D O_2 mapping

Planar optodes
Luminescent oxygen indicator
immobilised
in a hydrophobic matrix
on a transparent carrier

Fluorescence intensity imaging:
- need for O_2 permeable optical insulation
- non-transparent sensor foils

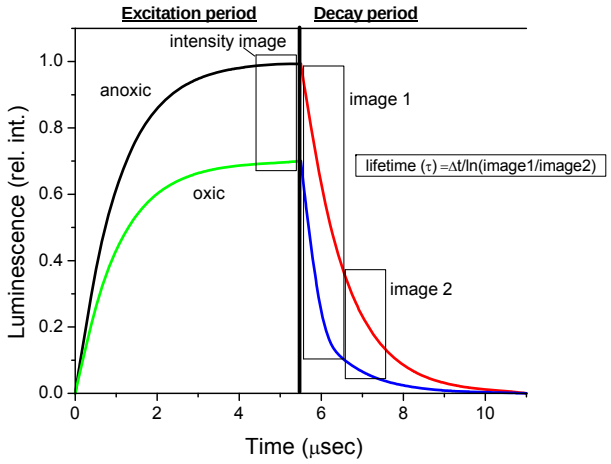
Fluorescence life-time imaging:
- transparent/semi-transparent sensor foils

Oxygen imaging



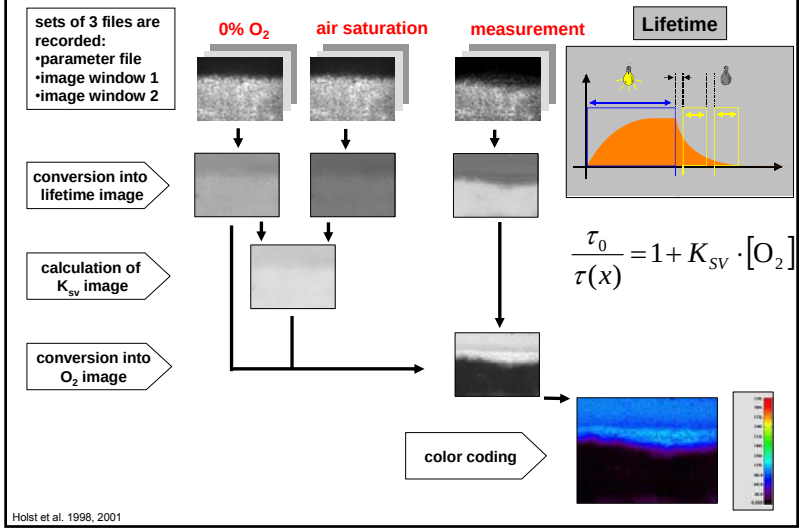
Glud et al. 1999

O_2 imaging: Intensity or life time based



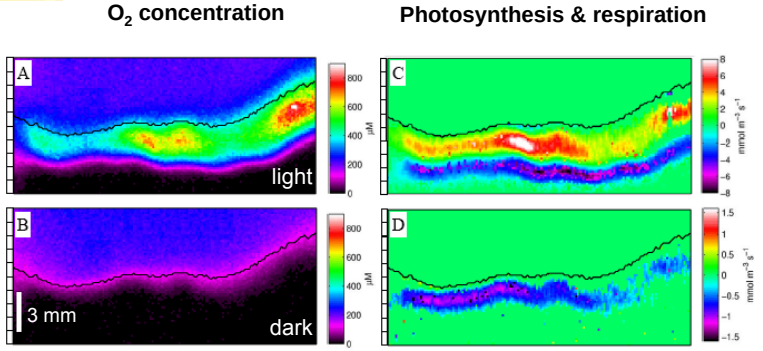
Holst, Kohls, Klimant, König, Richter, & Kühl (1998)

Image Processing



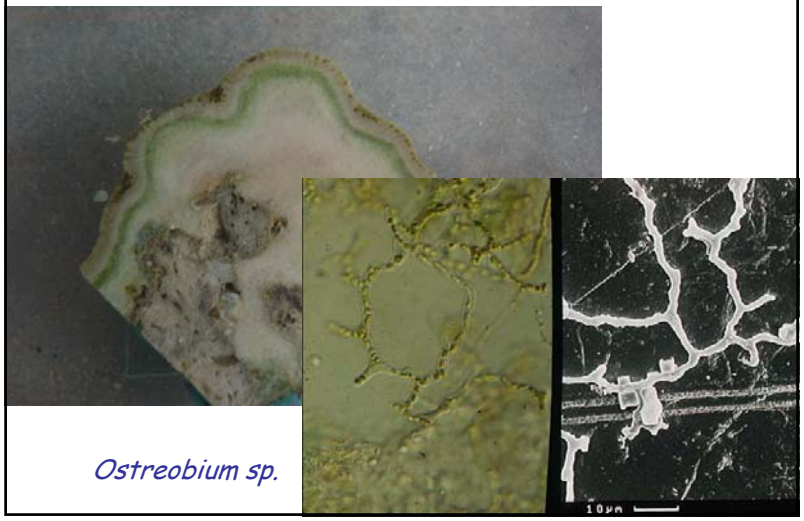
Holst et al. 1998, 2001

Oxygen and photosynthesis in a microbial mat



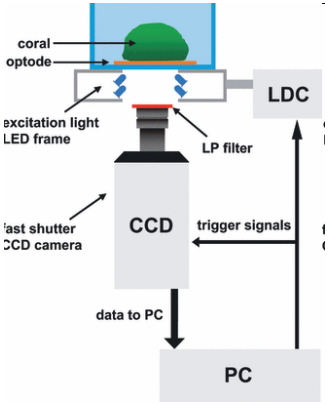
Kühl & Polerecky 2008

Endoliths in coral skeleton

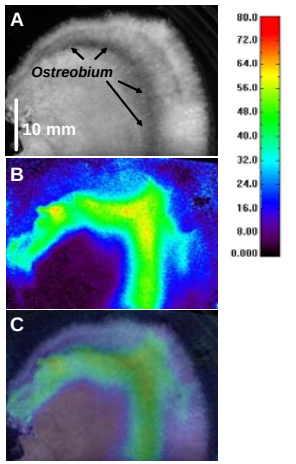


Ostreobium sp.

Endolithic biofilm in corals



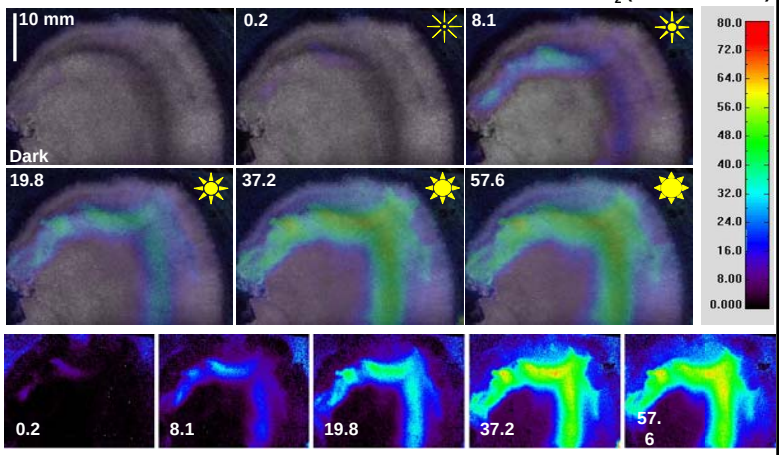
Oxygen (% air saturation)



Kühl, Holst, Larkum and Ralph 2008

Photosynthesis in endolithic biofilms

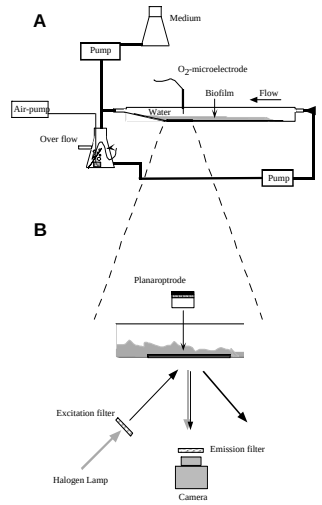
O_2 (% air saturation)



Kühl, Holst, Larkum and Ralph 2008

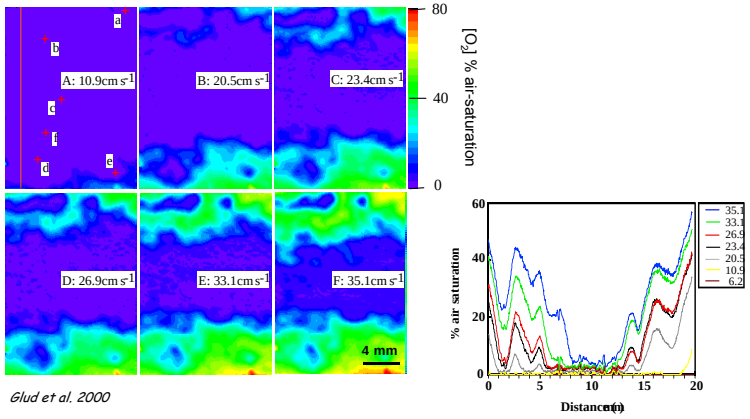
Biofilm reactor

Heterotrophic biofilm growing in complex organic medium on top of a planar oxygen optode.



Glud et al. 2000

Effect of flow on O_2 distribution at the basis of the biofilm

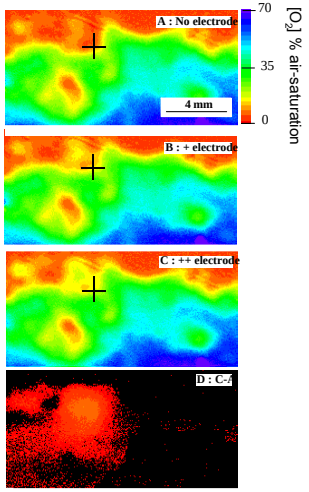


Glud et al. 2000

Effect of microsensor on O_2 distribution within biofilm

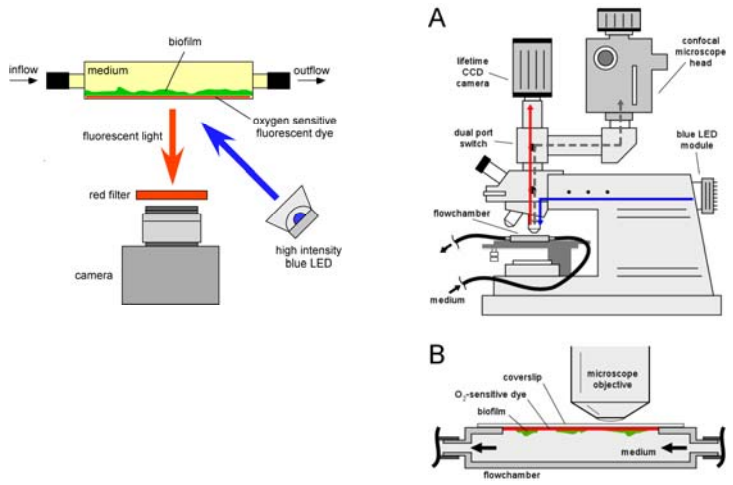
A microsensor was inserted in the biofilm at the cross.

The presence of the tiny microsensor tip disturbed the local oxygen microenvironment within the biofilm.

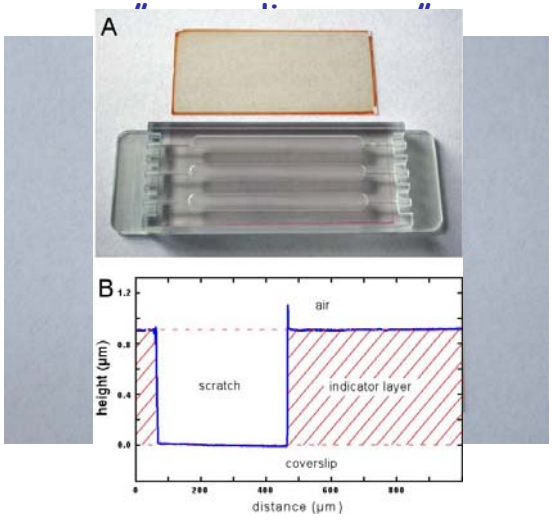


Glud et al. 2000

Flexible use with growth chambers



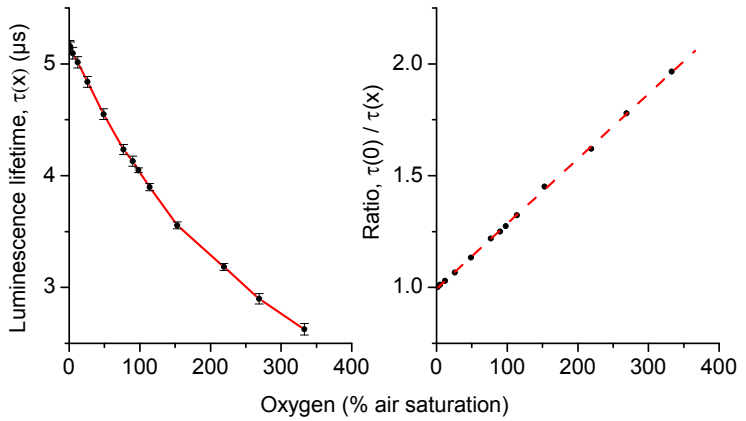
Planar oxygen nano-optode



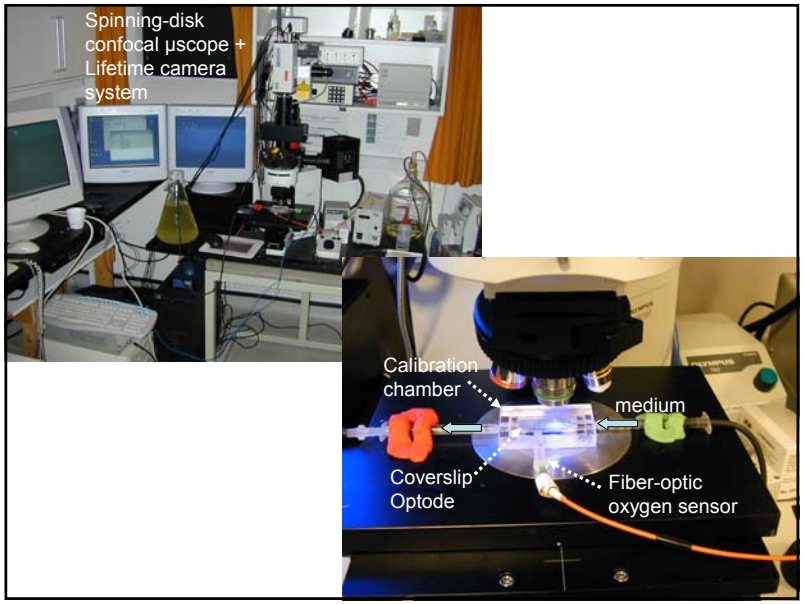
Kühl et al. 2007

Ideal Stern-Volmer quenching response

$$\frac{\tau_0}{\tau(x)} = 1 + K_{SV} \cdot [O_2]$$

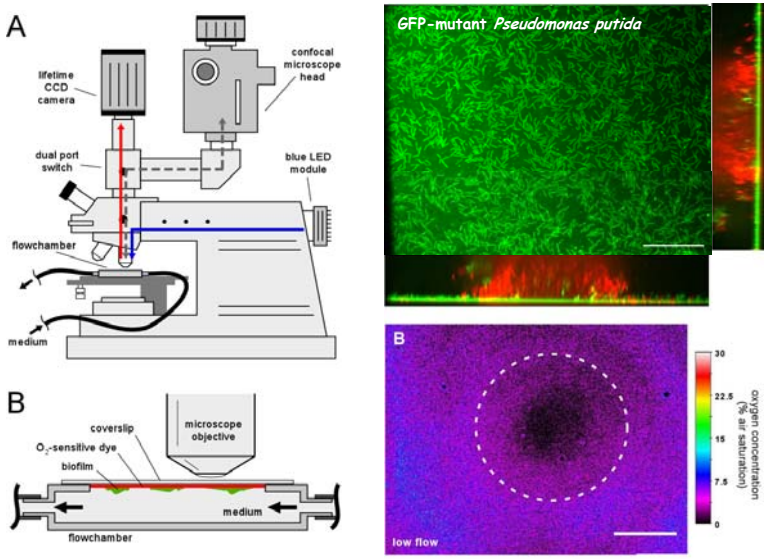


Kühl et al. 2007

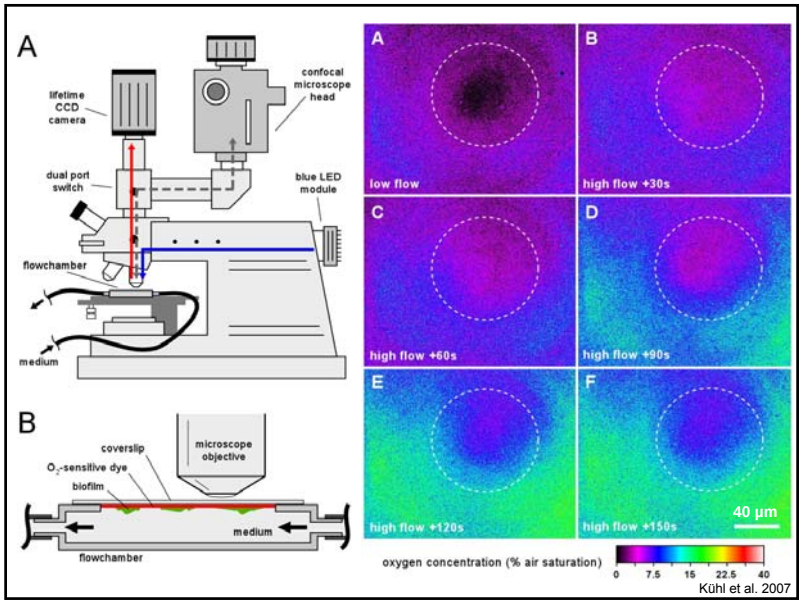


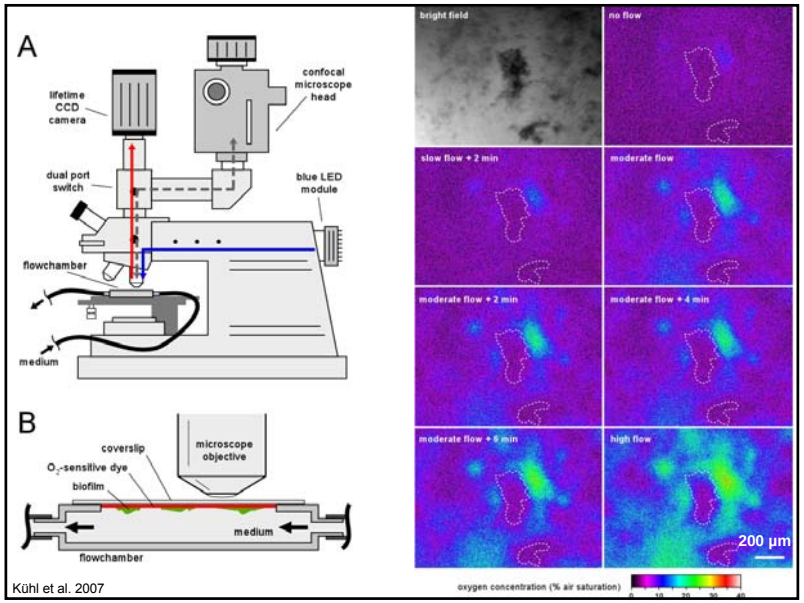
Flow-chamber biofilms growing on sensor

Kühl et al. 2007

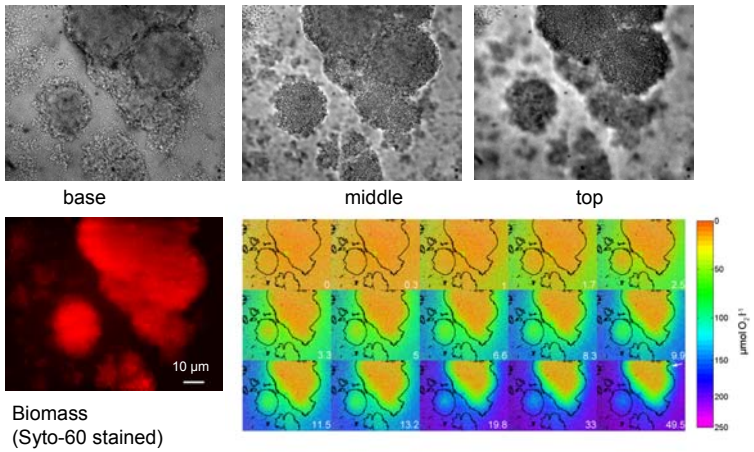


Kühl et al. 2007



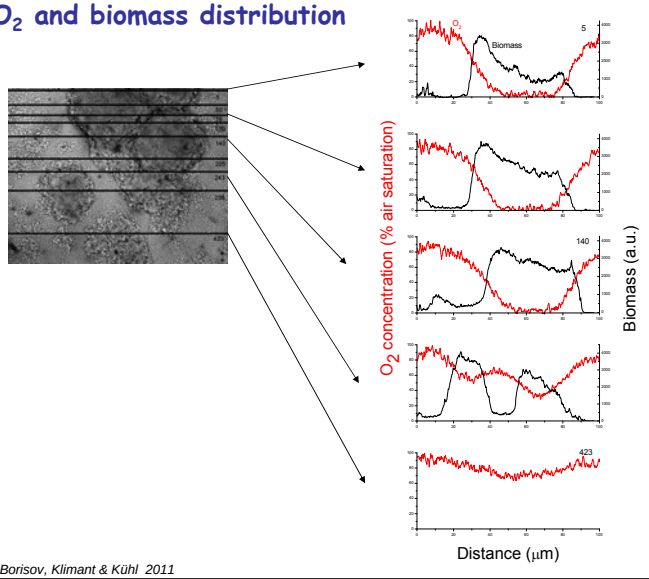


2D O_2 mapping at the base of an ~50 µm thick biofilm



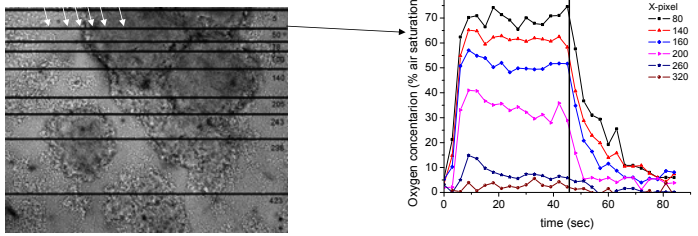
Stal, Borisov, Klimant & Kühl 2011

O_2 and biomass distribution

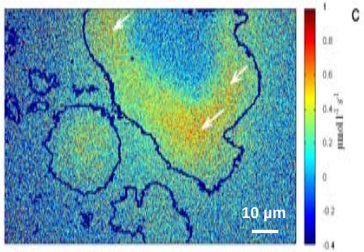


Stal, Borisov, Klimant & Kühl 2011

2D mapping of O_2 consumption



O_2 concentration over time after the flow was turned on and off.



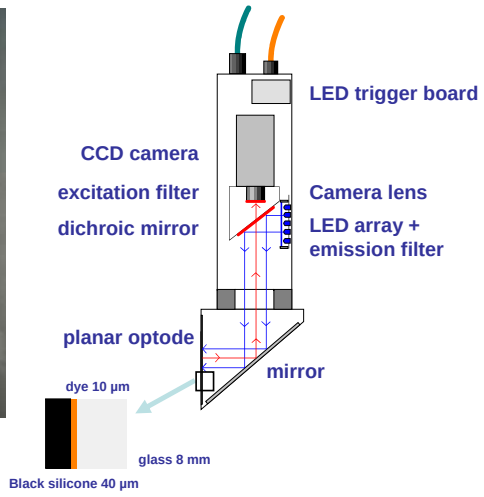
O_2 depletion rate after the flow was turned off

Stal, Borisov, Klimant & Kühl 2011

Microscopic O_2 imaging with planar nano-optodes

- Excellent tool for monitoring cell activity and biofilm growth on materials/surfaces.
- Combined imaging of chemical microenvironment, biofilm structure and GFP-expression in cells.
- Also useful for cell culture and tissue studies (e.g. test of biocides/antibiotics), materials science (e.g. biofouling prevention), and studies of many other surface-associated biological systems.

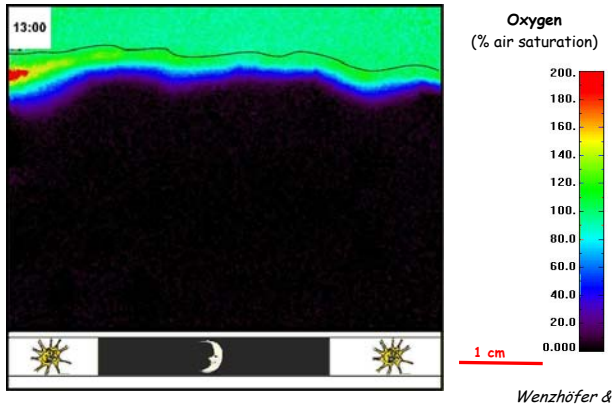
In situ planar optode module



Glud et al. (2001)

In situ oxygen dynamics

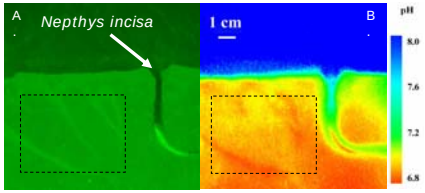
➢**24 hours time series in bioturbated sediment with microalgal biofilm** 13:00 – 13:00; 1 image every 15 min)



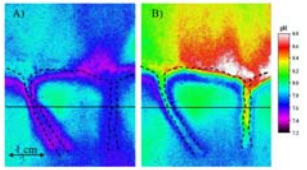
Wenzhöfer & Glud (2004)

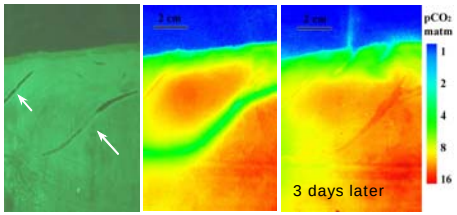
Planar sensors for other biogeochemical solutes

Zhu et al 2006 Environ Sci Tech 39:8906-11
Zhu et al 2006 GeoChim Cosmochi 19:4933-49



Stahl et al 2006 L&O Meth 4:336-45





Zhu et al 2006 Mar Chem 101:40-53

NH_4^+, H_2S, O_2/pH, ..Enzymes.. DGT/DET..SPI

Stromberg & Hulth 2005 Anal Chm 1-2:61-8
Robertson et al 2008 L&O Meth 6:502-512

Davison et al 1997 Nature 352:885-88
Davison et al 2000 IUPAC

Rhoads & Germano 1982 MEPS 25:253-94
Solan & Kennedy R 2002 MEPS 228:179-91

Rogers & Apte 2004 Environ Scien Tech 38:5134-40

Planar optodes

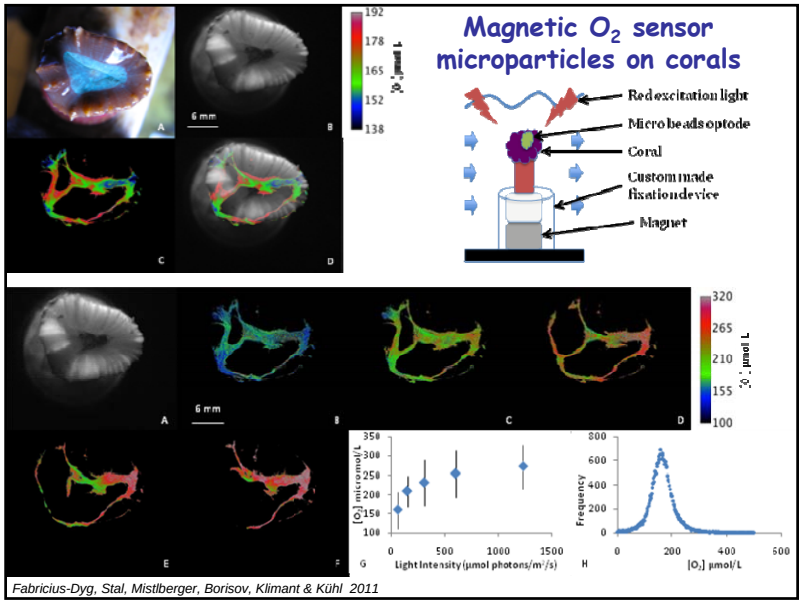
Advantages:

- ☺ Miniature instrument array, i.e. a sensor foil, with optical detection principle
- ☺ 2D- mapping of analyte with (1-)50-100 µm resolution over several (μm^2) cm^2
- ☺ Relative fast and reversible response (t_{90} <1-30 s)

Disadvantages:

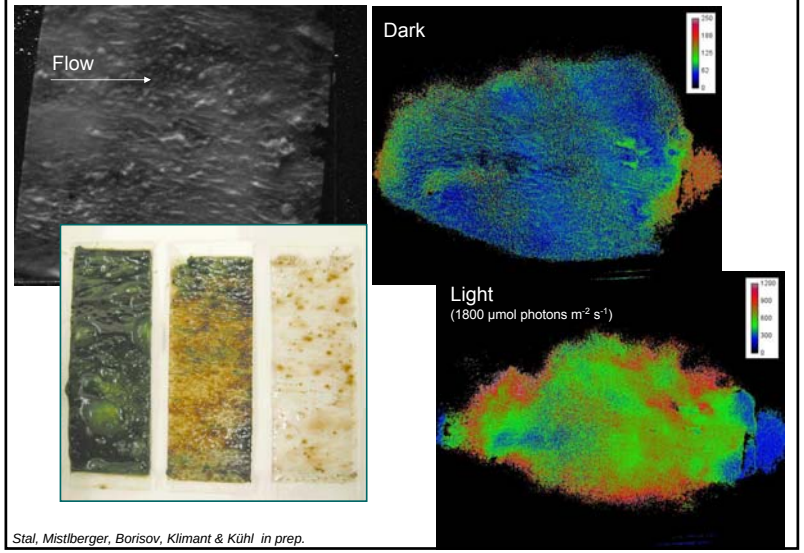
- ☹ Needs expensive imaging system and sophisticated software.
- ☹ **NOT** non-invasive!!!!
- ☹ Measurements at an "edge"
- ☹ Currently limited to O_2 , pH, NH_4^+ , CO_2 and temperature mapping in environmental applications

Magnetic O_2 sensor microparticles on corals



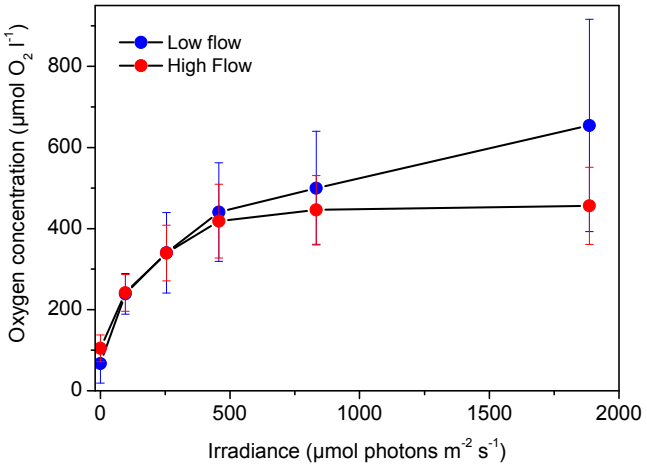
Fabricius-Dyg, Stal, Mistlberger, Borisov, Klimant & Kühl 2011

2D mapping of O_2 at the surface of biofilms



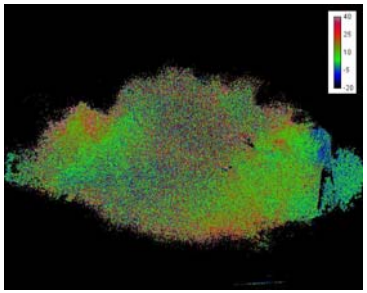
Stal, Mistlberger, Borisov, Klimant & Kühl in prep.

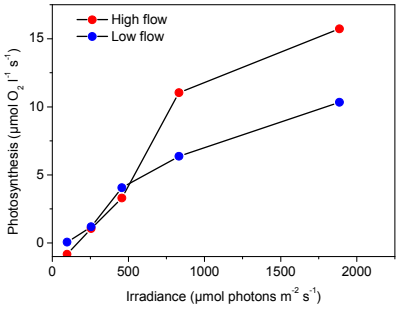
Mapping of O_2 vs. irradiance over the surface of a cyanobacterial biofilm



Stal, Mistlberger, Borisov, Klimant & Kühl in prep.

Mapping of gross photosynthesis over the surface of a cyanobacterial biofilm





O_2 depletion rate over first 2 seconds after darkening is taken as a proxy for gross photosynthesis

Stal, Mistlberger, Borisov, Klimant & Kühl in prep.